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Effect of H_3PO_4 Concentration and Particle Size of the Eggshell Used in Laying Hens Fed on Bone and Blood

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Abstract *- The objective of this research was to evaluate the effect of H_3PO_4 concentrations and particle size of eggshell used in the feed of laying hens on bone and blood profiles. Ninety-six laying hens (Isa Brown strain) age 25 weeks were reared in individual battery cage and divided into 8 groups randomly. Group 1 was fed using eggshell without H_3PO_4 and particle size of ≤1 mm (feed 1), group 2 was fed using eggshell that has been soaked in H_3PO_4 3% and particle size of ≤1 mm (feed 2), group 3 were fed using eggshell that has been soaked in H_3PO_4 4% and particle size of ≤1 mm (feed 3), group 4 was fed using eggshell that has been soaked in H_3PO_4 5% and particle size of ≤1 mm (feed 4), group 5 was fed using eggshell that without H_3PO_4 and particle size of ≤3 mm (feed 5), group of 6 was fed using eggshell that has been soaked in H_3PO_4 3% and particle size of ≤3 mm (feed 6), group 7 was fed using eggshell that has been soaked in H_3PO_4 4 % and particle size of ≤3 mm (feed 7) and a group of 8 was fed using eggshell that has been soaked in H_3PO_4 5% and particle size of ≤3 mm (feed 8). A Completely Randomized Design patterns factorial 4 x 2 x 3 was used in this research. Result of this research showed that had no interaction effect (P>0.05) between the H_3PO_4 concentration and particle size of eggshell on weight, volume, diameter of tibia bone and calcium and phosphorus content of the blood. The concentration of H_3PO_4 or particles size also had no effect (P>0.05) on all variables.*

Keywords — Egg shell, H_3PO_4, Particle Size, Bone and Blood

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I. INTRODUCTION

Development of the poultry industry have produced waste that can cause environmental pollution. Eggshell is one of the poultry industry waste but contain high calcium and little phosphorus. Besides that eggshell also contains micro mineral, protein, hormone and essential amino acid. Chicken eggshell powder contains Ca, P, Sr, Pb, Al, Cd, Hg, V, B, Fe, Zn, P, Mg, N, F, Se, Cu, and Cr (Schaafsma, et al. 2000). King'ori (2011) stated that eggshells can be utilized for various purposes that minimize their effect on environmental pollution. Eggshell powder has been reported to increase bone mineral density in people and animals with osteoporosis. According to Gongruttananun (2011) eggshell contains 34.89% calcium, 0.001% phosphorus and 5.35% protein. The use of coarse eggshell as substituted of fine limestone in the diet did not affect on plasma calcium and phosphorus content of the blood and tibia bone quality of RIR strain laying hens aged 38 weeks. Rovensky et al. (2003) stated that clinical and experimental studies indicate that eggshell powder has a positive effect on bone and cartilage that can be used for the prevention and treatment of osteoporosis.

The use of eggshell should be sterilized because it contains bacteria. The research of Kismiati et al. (2012) proved that soaking eggshell in the H_3PO_4 decrease the amount of bacteria and increase the phosphorus content. Meddleton and Ferket (2001) reported that the use of H_3PO_4 can kill bacteria and increase the phosphorus content of broiler carcass meal used as animal feed. Animal feed industry also uses H_3PO_4 to make monocalcium phosphate and dicalcium phosphate.

Phosphorus is the third most expensive nutrient after protein and energy. Phosphorus can be obtained from vegetable or animal feedstuffs. Phosphorus from animal and its products have higher availabilities than phosphorus from plant. Availability of phosphorus derived from plants approximately 30-40% (NRC, 1994) or 50% (North, 1978) and 30% (Larbier and Leclerq, 1994). This

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is caused phosphorus of plants bound with phytic acid that is not easily digested by birds, because birds do not have the phytase enzyme.

The research objective was to evaluate the effect of H_3PO_4 concentration and particle size of eggshell used in the feed on the bone and blood profile of laying hens. Utilization of eggshell in poultry feed expected contribute to minimize environmental pollution.

II. MATERIALS AND METHODS

The study begins with collecting the eggshell, soaking in hot water (80° C) for 15 minutes and then divided into 4 part. Part 1 without H3PO4, part 2 was soaked in H3PO4 3%, part 3 was soaked in H3PO4 4% and part 4 was was soaked in H3PO4 5% during 15 minutes. Each piece was milled with a filter size of 1 and 3 mm in order to obtain a particle size of <1 mm and <3 mm and then used as a mineral source of hens.

A total of 96 laying hens (Isa Brown strains) 25 weeks of age were used in this research and reared in individual battery cages for 12 weeks. Laying hens were divided into 8 groups randomly, so that each group consisted of 12 laying hens (4 hens/replication). Group 1 was fed using eggshell without H3PO4 and particle size of < 1 mm (Feed 1), group 2 was fed using eggshell that has been soaked in H3PO4 3% and particles size of < 1 mm (feed 2), group 3 was fed using eggshell that has been soaked in H3PO4 4% and particles size of < 1 mm (feed 3), group 4 was fed using eggshell that has been soaked in H3PO4 5% and particles size of < 1 mm (feed 4), group 5 was fed using eggshell without H3PO4 and particles size of < 3 mm (feed 5), group 6 was fed using eggshell that has been soaked in H3PO4 3% and particles size of < 3 mm (feed 6), group 7 was fed using eggshell that has been soaked in H3PO4 4% and particles size of < 3 mm (feed 7), group 8 was fed using eggshell that has been soaked in H3PO4 5% and particles size of < 3 mm (feed 8).

The composition of feed ingredients and nutrient content of feed research can be seen in Table 1. Proximate analysis of feed ingredients was used Wende methods (Tillman et al., 1986). Analysis of calcium and phosphorus contents of eggshell were determined by AAS method according to AOAC (1995) were cited in Hall et al. (2003).

Variables measured was the tibia bone profile (weight, volume, diameter, calcium and phosphorus content) and a blood profile (calcium and phosphorus content). Tibia profile measurements performed at the end of the study, while analysis of calcium and phosphorus in the blood done every 4 weeks. Completely randomized factorial design 4 x 2 x 3 was used in this research. Analysis of data used an analysis of variance and processed by Statistical Analysis Program (SAS).

III. RESULT AND DISCUSSION

Tibia Bone Profile

The effect of H_3PO_4 concentrations and particle size of the eggshell on tibia profile (weight, volume, and diameter) were presented in Table 2. The results of various analyzes indicate that there is no interaction effect between H_3PO_4 concentrations and particle size of eggshell on the tibia weight, volume, diameter, calcium and phosphorus content of the tibia bone.

Table 1. The Ingredients and Calculated Composition of the Experimental Feed

Feedstuff	Treatment feed							
	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
	%							
Corn	70.00	70.00	70.00	70.00	70.00	70.00	70.00	70.00
Soybean extract	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Poultry Meat Meal	11.00	11.00	11.00	11.00	11.00	11.00	11.00	1.00
Eggshell waste	5.00[a]	5.00[b]	5.00[c]	5.00[d]	5.00[e]	5.00[f]	5.00[g]	5.00[h]
Dicalcium Phosphate	1.10	1.00	0.80	0.75	1.10	1.00	0.80	0.75
Ca CO_3	2.40	2.50	2.70	2.75	2.40	2.50	2.70	2.75
Topmix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Na Cl	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
ME (kcal/kg)	2892.5	2892.5	2892.5	2892.5	2892.5	2892.5	2892.5	2892.5
Crude Protein (%)	16.45	16.45	16.45	16.45	16.45	16.45	16.45	16.45
Extract Ether (%)	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30
Crude fiber (%)	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70
Ca (%)	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
P *available* (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lycine (%)	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Methionine (%)	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44

[a]eggshell was sterilized with water of 80° C with a particle size of ≤1 mm, [b]eggshell was sterilized with water of 80° C and then soaked in 3% H_3PO_4 with particle size of ≤1 mm, [c]eggshell was sterilized with water of 80° C and then soaked in 4% H_3PO_4 with particle size ≤1 mm, [d]eggshell was sterilized with water of 80° C and then soaked in 5% H_3PO_4 and particle size of ≤1 mm, [e]eggshell was sterilized with water of 80° C and particle size ≤3 mm, [f]eggshell was sterilized with water of 80° C and then soaked in 3% H_3PO_4 and particle size ≤3 mm, [g]eggshell was sterilized with water of 80° C and then soaked H_3PO_4 4% and particle size of ≤3 mm, and [h]eggshell was sterilized with water of 80° C and then soaked in 5% H_3PO_4 and particle size ≤3 mm.

*Metionin, lisin, vitamin A, D_3,E , K, B_1, B_2, B_6, B_{12}, C, Ca-pantothenat, Niacin,Cholin Chloride Mn, Fe,I,Z,Co, Cu, Santoquin dan Zinc Bacitracin.

Profile of tibia bone (weight, volume, diameter, calcium and phosphorus content) had no significant different on different concentrations of H_3PO_4 (3-5%) and control. Onyango et al. (2003) and Fleming (2008) stated that bone mineral content correlated with mineral intake. Calcium and phosphorus are two most abundant mineral elements in bone (McDonald et al., 2002). Fard et al. (2010) stated that the use of calcium for bone formation. Bone calcium is mainly composed of calcium phosphate and some calcium carbonate. Feed of this studies contain a same of calcium and phosphorus despite concentrations of H_3PO_4 was different so weight, volume, diameter, calcium and phosphorus content of the tibia were not significantly different.

Soaking eggshell in H3PO4 decreases the amount of bacteria and increase the phosphorus content and concentration of H_3PO_4 (3 – 5%) had no effected on feed intake, calcium intake and phosphorus intake (Kismiati et al., 2012). Table 2 showed that the higher concentration of H_3PO_4 caused DCP in feed decreases. Results of this research indicated that the phosphorus of H_3PO_4 can replace phosphorus from DCP.

Table 2. Tibia bone profile of laying hens fed using eggshell soaked in H_3PO_4 and different particle sizes.

Variable	Particle size (mm)	Concentation of H_3PO_4 (%)				Means[ns]
		0	3	4	5	
Weight (g)	≤1	9.47	9.33	9.57	9.34	9.43
	≤3	9.65	9.66	9.45	9.92	9.67
Means[ns]		9.56	9.49	9.51	9.63	
Volume (cc)	≤1	6.70	6.73	7.10	6.93	6.86
	≤3	7.16	7.17	7.20	7.13	7.14
Means[ns]		6.93	6.95	7.10	7.03	
Diameter (cm)	≤1	0.58	0.60	0.59	0.56	0.58
	≤3	0.59	0.58	0.60	0.58	0.59

[ns] Non significant (P>0,05)

Particle size of eggshell had no significant effect (P>0.5) on the bone profile (bone weight, bone volume and bone diameter). Profile tibia bone did not differ due to

differences in particle size is very small. Pizzolante *et al*. (2009) stated that the use of limestone particle size of 2-5 mm and > 5mm increasing calcium tibia bone. Saunders-Blades et al. (2009) examined the effect of particle size of oyster shell, white limestone, red limestone and gray limestone on bone quality and result that a higher weight on the use of a mixture of 1/3 of small particles + 2/3 larger than the size of small particles, but the calcium content was not significantly different. The results of this research correspond with the study of de Witt et al. (2009) that the particle size did not effect on weight and diameter of tibia bone of laying hens.

The average weight of tibia bone 7.41 to 9.92 g, the volume of 6.93 to 7.10 cc, while the particle size based, the weight of tibia 7.41 to 7, 47 g and volume 6.86 to 7.14 cc. Tibia weight lower than the study of Rath et al., (2000), while the volume in the normal range. Volume of tibia bone is 7 to 7.17 cc (Zang and Coon, 1997) and tibia weight is 18.56 g (Rath et al., 2000).

Calcium and Phosphorus of Blood

Calcium and phosphorus content of the blood can be seen in Table 3. Blood calcium and phosphorus did not influenced by the interaction of H_3 PO_4 concentration and particle size. Concentration of H_3 PO_4 or particle size did not significant effect on blood calcium and phosphorus.

Table 3. Blood calcium and phosphorus of laying hens fed using eggshell soaked in H_3PO_4 and different particle sizes.

Variable	Particle size (mm)	Concentation of H_3PO_4 (%)				Means[ns]
		0	3	4	5	
		mg/dl				
Calcium	1	24.,38	21.91	25.18	24.03	23.87
	3	24.76	27.21	24.75	24.59	25.32
Means[ns]		24.57	24.56	24.96	24.31	
Phosphorus	1	7.36	7.41	7.55	7.30	7.41
	3	7.39	7.56	7.35	7.56	7.46
Means[ns]		7.37	7.48	7.45	7.43	

[ns] Non significant (P>0,05)

Calcium and phosphorus of the blood were not significantly different at the different H_3PO_4 concentrations. Research using the feed with the same content of calcium and phosphorus although concentrations of H_3PO_4 differently so the blood calcium and phosphorus were not significantly different. According to Celebi et al. (2005) blood phosphorus correlated with phosphorus of the feed. Pelicia et al. (2009) stated that calcium blood concentration is affected by calcium level of the feed. The study of Fard et al. (2010) showed that the decrease and increase in calcium and phosphorus feed 10% of 0.37% does not increase or decrease blood calcium and phosphorus of the blood.

Eggshell particle size used in the feed had no effect on calcium and phosphorus of blood caused by differences particle size can be tolerated in the mechanism of absorption of calcium and phosphorus. According to McNab and Boorman (2002) Calcium and phosphorus from feed digested in the proventriculus and ventrikulus then absorbed by the blood in the intestinal/intestines. Absorption was determined to be most efficient from the duodenum to the upper jejunum. Elble et al. (2009) stated that the absorption of calcium from the small particle size of $CaCO_3$ is lower than the large particle size. According Pizzolante et al. (2006), the smaller particle size the more easily absorbed then larger. Pelicia et al. (2009) reported that the particle size of limestone 0.18 and 3.13 mm did not significantly affect on blood calcium concentrations of laying hens aged 58 weeks. Pelicia et al. (2011) examined the use of limestone particle size of 0.18 and 3.90 mm at strain Isa Brown hens aged 23 weeks. The results showed that the particle size of limestone 0.18 and 3.90 mm had no effect on blood calcium although the solubility *in vitro* particle size 0.18 mm higher than 3.90 mm. Azar et al. (2011) investigated the use of rock/perlite particle size of 1.5 and 3 mm as a mineral resource of broilers aged 42 days and obtained the result that the concentration of blood serum calcium and phosphorus did not differ.

IV. CONCLUSIONS

The conclusion of this study is H_3PO_4 concentrations used for soaking the eggshell did not effect on the tibia bone profile and blood profile, as well as particle size.

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